Comparative analysis of antioxidant enzyme activities in non-diapause, summer diapause and winter diapause pupae of *Delia antiqua* (Diptera: Anthomyiidae)

HAO You-Jin*, GUO Qiang, CHEN Bin

(Chongqing Key Laboratory of Vector Insects, Institute of Entomology and Molecular Biology,
Chongqing Normal University, Chongqing 401331, China)

Abstract:【Aim】In order to investigate the differences of antioxidant system in non-diapause (ND), summer diapause (SD) and winter diapause (WD) pupae of *Delia antiqua*, the variations of antioxidant enzyme activities and glutathione redox status were determined and compared.【Methods】Time-course variations of antioxidant enzyme activities of copper/zinc-SOD (Cu/Zn-SOD), manganese superoxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), and contents of reduced glutathione (GSH) and oxidized glutathione (GSSG) and their ratio were measured and compared between different stages of ND, SD and WD pupae of *D. antiqua*. Canonical discriminate analysis was also conducted to further explore the relationships between the major antioxidant components.【Results】The activities of all the five antioxidant enzymes dynamically changed during the whole pupal stage. Compared with ND and SD pupae, WD pupae at the pre-diapause phase had higher Cu/Zn-SOD activities. The mean Cu/Zn-SOD activity in SD or WD pupae at the diapause maintenance phase and post-diapause phase was much lower than that in ND pupae. In the whole pupal stage, ND pupae had higher MnSOD activities than SD and WD pupae, but no difference existed between SD and WD pupae. The average MnSOD activity was distinctly higher than that of Cu/Zn-SOD at the same developmental stage. The CAT activities in SD and WD pupae at the pre-diapause phase were statistically higher than that in ND pupae at the same developmental stage, however, the CAT activities in SD or WD pupae at the diapause maintenance phase and post-diapause phase were significantly lower than those in ND pupae. In either non-diapause or diapause pupae at the same developmental stage, the general GPx activities showed an opposite variation trend with GR activities. Canonical discriminant analysis revealed that antioxidant system in onion fly pupae showed diapause type specificity.【Conclusion】Redox status in ND pupae is significantly different from that in SD and WD pupae. Higher antioxidant enzyme activities and lower GSH/GSSG ratio in pupae at the pre-diapause phase and post-diapause phase suggest that the oxidative shift results from a higher respiration rate and development changes.

Key words: *Delia antiqua*; diapause; antioxidant enzyme; oxidative stress; glutathione redox cycle

1 INTRODUCTION

Diapause is a specific state of physiological dormancy and characterized by the accumulation of energy reserves, metabolic depression, and enhanced stress tolerances (MacRae, 2010). Metabolism is decreased remarkably during the diapause process, but it isn't entirely inhibited. Reactive oxygen species (ROS) are still continuously generated mainly through the mitochondrial respiratory chain. Excessive ROS generation would cause oxidative stresses, disturb the homeostasis, and even cause cellular death (Rajarapu *et al.*, 2011). However, ROS also stimulate signal transduction and mediate various responses including development, cell growth, metabolism and defense of cells (Hernández-García *et al.*, 2010).

To reduce oxidative damages to cellular components, insects have evolved antioxidant

---

基金项目：重庆市科委项目(cstc2016jcyjA0152)；“两江学者”计划专项经费(20136666)；国家自然科学基金项目(31372265)；国际原子能机构 IAEA 研究项目(18268/R2)

作者简介：郝友进，男，1973 年 7 月生，山东莒县人，博士，教授，研究方向昆虫发育分子生物学，E-mail：haoyoujin@hotmail.com

* 通讯作者 Corresponding author, E-mail：haoyoujin@hotmail.com

收稿日期 Received：2017-10-06；接受日期 Accepted：2017-12-15

systems to effectively eliminate ROS using enzymatic and non-enzymatic system or maintain a tolerable level (Dmochowska-Ślęzak *et al*., 2015). The main antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). SOD converts superoxide radical anions (O_2^{-}) to hydrogen peroxide (H_2O_2), which is then decomposed to water by CAT or GPx (Costantini, 2014). Accordingly, factors that affect antioxidant enzyme activities would result in the accumulation of ROS and might cause oxidative damages. In *Caenorhabditis elegans*, the overexpression of the cytosolic Cu/Zn-SOD gene (*sod*-1) could increase life span by activating longevity-promoting transcription factors, but not by the removal of (O_2^{-}) (Cabreiro *et al*., 2011). Similarly, the expression level of Cu/Zn-SOD gene (*sod*-2) was remarkably increased in diapause *Culex pipiens* females than in non-diapause counterparts (Sim and Denlinger, 2011). In insects, CAT is considered as the key enzyme to be responsible for the scavenging of ROS (Nabizadeh and Kumar, 2011). Suppression of CAT by RNAi would increase damages to the ovaries of *C. pipiens*, suggesting it was particularly important for the survival of diapause *C. pipiens* females (Sim and Denlinger, 2011). GPx catalyzes the reduction of H_2O_2 and lipid hydroperoxides using glutathione (GSH) as the cosubstrate. The removal of hydroperoxides is accompanied by oxidation of GSH to glutathione disulfide (GSSG), whose reduction back to GSH is catalyzed by GR (Missirlis *et al*., 2001; Munks *et al*., 2005; Mittapalli *et al*., 2007). GSH has been shown to be an important component of the ROS signaling pathways in regulating GSH levels and oxidation state in *Bombyx mori* (Zhao *et al*., 2014).

The onion fly, *Delia antiqua*, is a major underground pest attacking onions and closely related *Allium* species globally, and an emerging model for pupal diapause researches (Hao *et al*., 2016). Onion fly can enter into pupal diapause in response to long-day photoperiod in summer and short-day photoperiod in autumn to survive unfavorable seasonal changes to their habitats. Although enhanced stress tolerance is a highly conserved feature of diapause, it is unclear the extent to which responses in antioxidant systems during the diapause process. In this study, the activities of Cu/Zn-SOD, MnSOD, CAT, GPx and GR, and the contents of GSH and GSSG and their ratio were determined in diapause and non-diapause pupae of *D. antiqua* to explore the changes and the role of antioxidant components during diapause of this insect.

2 MATERIALS AND METHODS

2.1 Test insects

Laboratory colonies of *Delia antiqua* was maintained and reared on an artificial diet in our laboratory as previously described (Fu *et al*., 2016; Hao *et al*., 2016). Non-diapause (ND) larvae were reared at 20℃ ±0.5℃ under a 16L∶8D photoperiod and 50% –70% relative humidity. Summer diapause (SD) pupae were achieved by rearing the larvae at 25℃ ±0.5℃ under a 16L∶8D photoperiod with 50% –70% relative humidity. Winter diapause (WD) pupae were achieved by rearing the larvae at 15℃ ±0.5℃ under a 12L∶12D photoperiod with 50% –70% relative humidity. Pupae were collected according to the developmental stage as illustrated in Fig. 1.

2.2 Antioxidant enzyme activity assays

To determine the activities of antioxidant enzymes, three pupae of each developmental stage were homogenized in 0.5 mL ddH_2O, centrifuged at 12 000 × g 4℃ for 5 min, and the supernatants were used for enzymatic assays and protein concentration measurement. Total soluble protein concentration of each sample was determined using the Bradford method (Bradford, 1976) with bovine serum albumin as a protein standard. All enzyme activities were determined spectrophotometrically using the Kits (Beyotime, China).

Total SOD activity was measured by the inhibition of superoxide radical production using the WST-1 method (De Block and Stoks, 2008). In a 96-well microtiter plate, 20 μL of enzyme extract was mixed with 20 μL working solution and 180 μL of WST. After incubation at 37℃ for 20 min, the absorbance at 450 nm was measured. One unit of SOD activity was defined as the amount of enzyme needed to inhibit 50% xanthine oxidase activity. The activity of Cu/Zn-SOD was determined by subtracting the MnSOD value from the total SOD value.

CAT activity was measured by the rate of hydrogen peroxide decomposition at 520 nm. In a 96-well microtiter plate, 20 μL enzyme extract was mixed with 20 μL PBS buffer (pH 7.0), and then 10 μL of H_2O_2 (5 mmol/L final concentration) was added and incubated at 25℃ for 5 min, then 450 μL stop solution was added to stop the reaction. Ten μL above products and 200 μL of chromogenic solution were mixed, incubated at 25℃ for 15 min and the absorbance at 520 nm was measured. One unit of CAT activity was defined as the amount of enzyme required to catalyze 1 μmol of H_2O_2 per min.

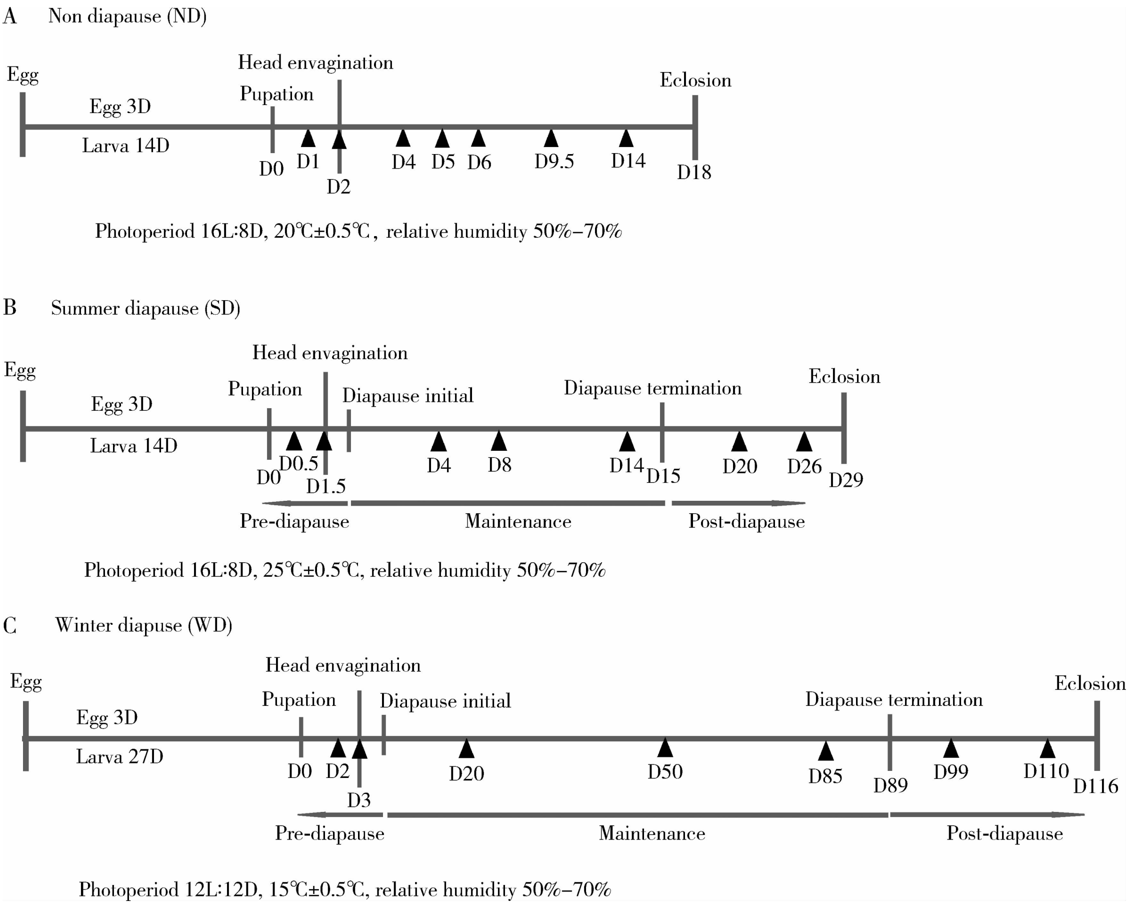

Fig. 1 Pupal diapause induction of *Delia antiqua* and experiment designing

Pupal period was calculated from the day of pupation (D0) to adult emergence. The pupal period of non-diapause (ND), summer diapause (SD) and winter diapause (WD) was about 18, 29 and 116 days, respectively. Pupae were collected (marked by triangle symbols) according to the developmental stage after pupation. Developmental stage 1: Before the head envagination (ND1 = SD0.5 = WD2); Developmental stage 2: Head envagination completed (ND2 = SD1.5 = WD3); Developmental stage 3 (ND4 = SD4 = WD20) and stage 4 (ND5 = SD8 = WD50): Free fat body appears in the central area of eyes; Developmental stage 5: Yellow body appears (ND6 = SD14 = WD85); Developmental stage 6: Yellow body is moving (ND9.5 = SD20 = WD99); Developmental stage 7: Body is initially covered with yellow bristles (ND14 = SD26 = WD110).

GPx catalyzes the oxidation of glutathione in the presence of hydroperoxide, which is then reduced by glutathione reductase with NADPH as the coenzyme. The reaction mixture (200 μL) consisted of 10 μL working solution (10 mmol/L NADPH, 84 mmol/L GSH and GR), 10 μL enzyme extraction, 176 μL PBS buffer (pH 7.0), and 4 μL H_2O_2 (15 mmmol/L final concentration). The decrease in NADPH absorbance at 340 nm was measured. One unit of GPx activity was defined as the amount of enzyme required to oxidize 1 mmol NADH to $NADP^+$ per minute at 25℃.

The reaction mixture for GR activity contained 100 μL GSSG (2 mmmol/L final concentration), 70 μL Tris-HCl buffer (pH 7.5), 20 μL enzyme extract and 10 μL NADPH (2 mmmol/L final concentration). The decrease of absorbance at 340 nm was monitored at 25℃ for 5 min. One unit of GR activity was defined as the amount of enzyme required to catalyze the oxidation of 1 μmol NADPH per min.

2.3 Determination of GSH and GSSG contents

The total contents of glutathione (GSH) and oxidized disulfide GSSG were measured using an assay Kit (Beyotime, China). Briefly, the mixture containing 5 μL enzyme extraction, 5 μL protein remover M and 150 μL detecting buffer was incubated at 25℃ for 5 min and then 50 μL NADPH (0.16 mg/mL) was added. The total glutathione content was measured by the absorbance at 412 nm. The GSSG activity was assayed in two steps: 100 μL enzyme extraction and 5 μL GSH solution were mixed, then 1 μL GSH remover solution was added and incubated at 25℃ for 60 min. The second reaction was used as the total glutathione detection, and the GSSG content could be obtained. The reduced glutathione content was determined by subtracting the GSSH content from the total glutathione content.

2.4 Statistical analysis

All data were represented as mean with

standard error (*SE*) (n = 3 × 3) with triplicate and three biological replicates. The difference within the same group was compared by one-way analysis of variance (ANOVA) and means were compared with Tukey's Studentized range (HSD) test. The effect of developmental stages on the antioxidant enzyme activities was calculated by two-way ANOVA. Canonical discriminate analysis was performed to explore the coordination of antioxidant enzyme between different pupal groups. Differences were considered statistically significant when $P < 0.05$. All statistical analyses were performed using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3 RESULTS

3.1 Time-course variation of enzyme activities in ND, SD and WD pupae

The Cu/Zn-SOD activities varied non-uniformly in ND, SD and WD pupae (Fig. 2: A). Cu/Zn-SOD activities in WD pupae at the pre-diapause phase (developmental stage 1 and 2) (0.45 ± 0.08 – 0.62 ± 0.01 U) were significantly higher than those in ND pupae (0.32 ± 0.09 – 0.38 ± 0.08 U) and SD pupae (0.18 ± 0.08 – 0.23 ± 0.1 U); however, the average Cu/Zn-SOD activity in ND pupae at developmental stages 4 – 7 (0.31 ± 0.05 – 0.62 ± 0.1 U) was much higher than those in SD pupae (0.16 ± 0.07 – 0.34 ± 0.04 U) and WD pupae (0.05 ± 0.03 – 0.22 ± 0.08 U). Higher MnSOD activities were observed in ND pupae (0.70 ± 0.08 – 1.28 ± 0.3 U) than in SD (0.25 ± 0.07 – 0.62 ± 0.08 U) and WD pupae (0.31 ± 0.09 – 0.69 ± 0.05 U). Moreover, the average MnSOD activity was distinctly higher than that of Cu/Zn-SOD at the same developmental stage (Fig. 2: A and B). However, MnSOD activity was not different between SD pupae and WD pupae at the pre-diapause phase (developmental stage 1 and 2). The CAT activities in diapause pupae were higher than those in non-diapause pupae at the developmental

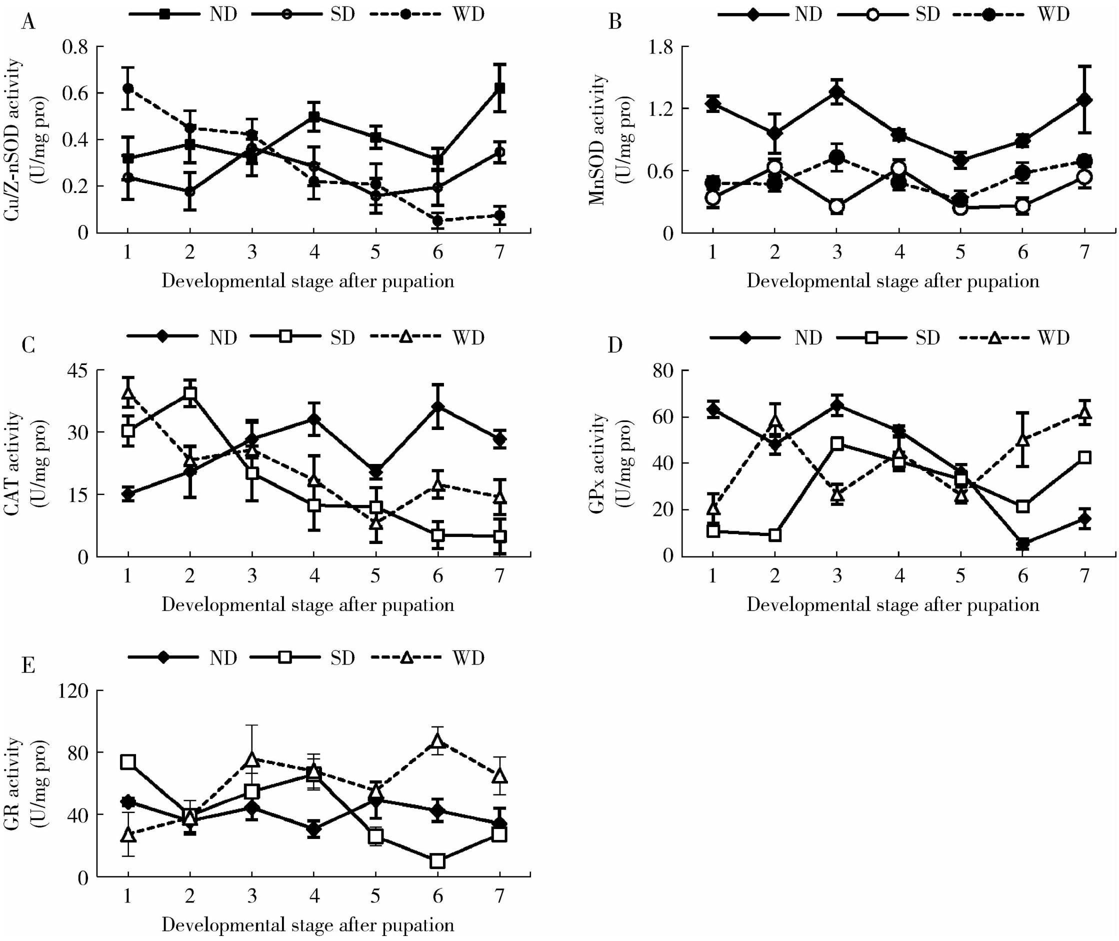

Fig. 2 Variations of the Cu/Zn-SOD (A), MnSOD (B), CAT (C), GPx (D) and GR (E) activities in non-diapause (ND), summer diapause (SD) and winter diapause (WD) pupae of *Delia antiqua*

1: Before the head envagination (ND1 = SD0.5 = WD2); 2: Head envagination completed (ND2 = SD1.5 = WD3); 3: Free fat body appears in the central area of eyes (ND4 = SD4 = WD20); 4: Free fat body appears in the central eyes (ND5 = SD8 = WD50); 5: Yellow body appears (ND6 = SD14 = WD85); 6: Yellow body is moving (ND9.5 = SD20 = WD99); 7: Body is initially covered with yellow bristles (ND14 = SD26 = WD110). The same is for Fig. 3. The effect of developmental stages on the antioxidant enzyme activities was calculated by one-way ANOVA. Bars indicate standard errors. Values are means ± *SE* of three independent experiments with triplicates in each experiment (n = 3 × 3).

stage 1 (SD: 30.3 ± 3.7 U; WD: 39.5 ± 3.7 U; ND: 15.1 ± 1.7 U) ($F = 46.7$, $P = 0.000$) and stage 2 (SD: 39.4 ± 1.2 U; WD: 23.3 ± 3.2 U; ND: 20.5 ± 6.2 U) ($F = 18.6$, $P = 0.003$). Similar to Cu/Zn-SOD activities, the CAT activities in ND pupae at developmental stages 4 – 7 (20.4 ± 1.7 – 36.1 ± 5.3 U) were significantly higher than those in SD (4.9 ± 1.1 – 20.1 ± 2.5 U) and WD pupae at the same stage (8.1 ± 4.7 – 25.8 ± 6.5 U) (all $P < 0.01$) (Fig. 2: C). For both SD and WD pupae, their CAT activities decreased gradually during the diapause process.

At the diapause maintenance phase (developmental stages 3 – 4), the GPx activities in both SD and WD pupae were lower than that in ND pupae (all $P < 0.01$), but at the post-diapause phase, the GPx activities in both SD and WD pupae were significantly higher than those in ND pupae (developmental stages 6 – 7) (Fig. 2: D) (all $P < 0.01$). Generally, the change trend of GPx activities was opposite to that of Cu/Zn-SOD activities. And the GR activities showed an opposite change trend with GPx activities in ND, SD and WD pupae (Fig. 2: E).

3.2 GSH and GSSG contents in ND, SD and WD pupae

To better understand the variation of the glutathione redox status in ND, SD and WD pupae of *D. antiqua*, the contents of GSH and GSSG and the GSH/GSSG ratio were measured. As shown in Fig. 3 (A), no significant differences in GSH content were seen not only within the groups of ND ($F = 0.28$,

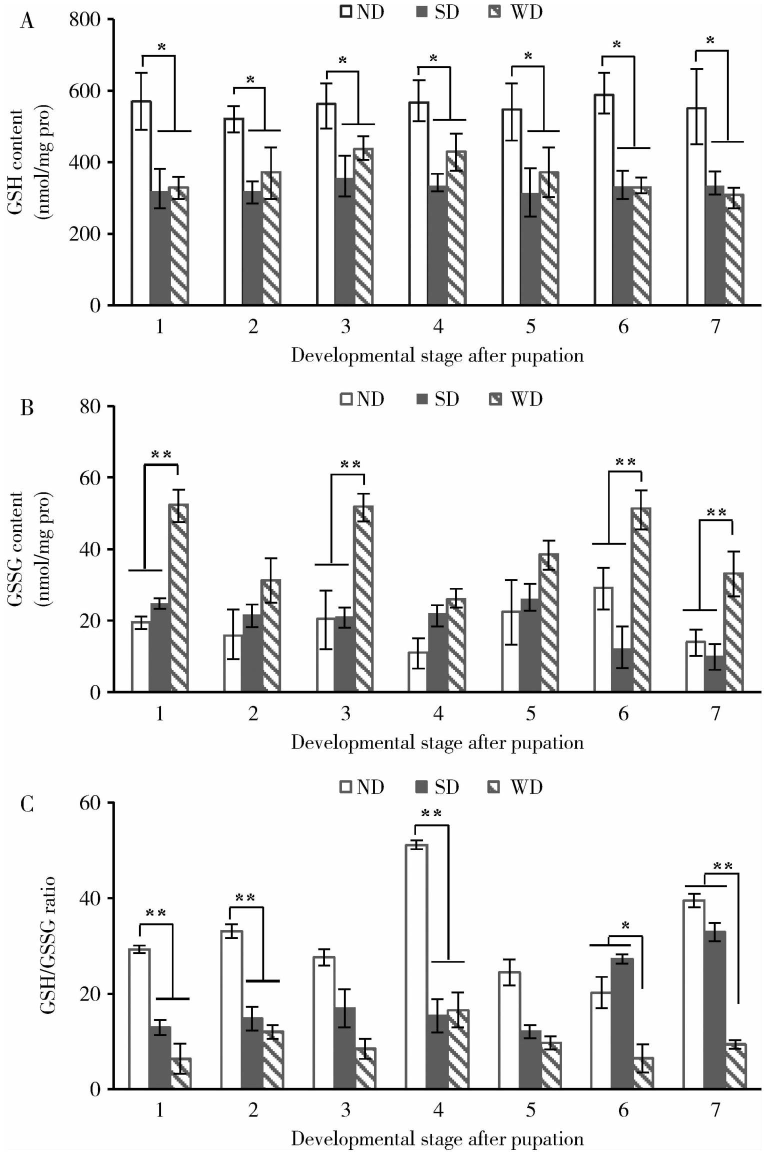

Fig. 3　Variations of GSH content (A), GSSG content (B), and GSH/GSSG ratio (C) between non-diapause (ND), summer diapause (SD) and winter diapause (WD) pupae of *Delia antiqua*

Data were expressed as means ± *SE* of three independent experiments with triplicates in each experiment (n = 3 × 3). The difference within the same developmental stage was compared by one-way analysis of variance (ANOVA). Significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$.

$P=0.94$), SD ($F=2.50$, $P=0.07$) and WD pupae ($F=3.14$, $P=0.84$), respectively, but also between SD and WD pupae at the same developmental stage (all $P>0.05$). Interestingly, the GSH content in ND pupae at each developmental stage was higher than that in SD or WD pupae (Fig. 3: A) (all $P<0.05$). GSSG content varied significantly within each group (ND: $F=3.16$, $P=0.04$; SD: $F=3.44$, $P=0.03$; WD: $F=4.936$, $P=0.01$) (Fig. 3: B). The variation in GSSG content in WD pupae generally showed a Z-shaped pattern, and higher GSSG content occurred at the pre-diapause phase (stage 1), maintenance phase (stage 3), and post-diapause phase (stage 6 and stage 7). Additionally, the GSSG content in WD pupae was significantly higher when compared with that in ND pupae or SD pupae at the same developmental stage (for stage 1: $F=78.99$, $P=0.000$; stage 3: $F=31.02$, $P=0.001$; stage 6: $F=13.09$, $P=0.02$ and $F=4.46$, $P=0.03$). However, no significant differences were observed between ND and SD pupae at the same developmental stage. In ND pupae, the GSH/GSSG ratio also varied significantly during the pupal period ($P<0.01$), but was higher at stages 2, 4 and 7 and peaked at stage 4 (Fig. 3: C). The GSH/GSSG ratios in SD pupae at the post-diapause phase (stages 6 and 7) were higher than those at the pre-diapause and maintenance phases. Interestingly, no significant differences were found within WD group ($P>0.05$).

3.3 Coordination of antioxidant enzymes

To further explore the relationship of different enzymes, canonical discriminant analysis was performed. The mathematical expression of this analysis is a canonical discriminant function with its own significant: $Y=0.992\times\text{MnSOD}+0.158\times\text{Cu/Zn-SOD}+0.01\times\text{CAT}-0.037\times\text{GP}_\text{X}-0.29\times\text{GR}$ ($P<0.01$). This discriminant function highlighted the difference between ND, SD and WD pupae, and elegantly described anti-oxidative defense by MnSOD (standardized canonical discriminant function coefficient = 0.992) and GR (standardized canonical discriminant function coefficient = −0.29) as the most important discriminative variables (Fig. 4).

Our results also showed that the enzyme activities varied significantly among ND, SD and WD pupae: Cn/Zn-SOD ($F=2.12$, $P<0.05$), MnSOD ($F=21.97$, $P<0.05$), CAT ($F=0.91$, $P<0.05$), GP_X ($F=2.209$, $P<0.05$) and GR ($F=1.10$, $P<0.05$). Enzyme activities were significantly correlated with pupal developmental stage and diapause status (SD or WD) ($F=4.373$, $P<0.05$). Furthermore, developmental stage significantly affected the activities of Cu/Zn-SOD, CAT, GPx and GR, but not those of MnSOD in ND, SD and WD pupae.

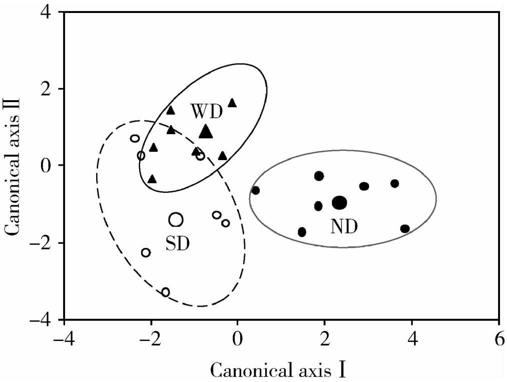

Fig. 4 Two-dimensional discriminant analysis showing the differences in activities of antioxidant defense enzymes in non-diapause (ND), summer diapause (SD) and winter diapause (WD) pupae of *Delia antiqua*

Smaller triangles, black dots and circles represent different antioxidant enzymes in WD, ND and SD pupae, while the larger triangle, black dot and circle represent the centroid of each group, respectively.

4 DISCUSSION

High or low temperature affects almost all biological processes, including the rate of physiological and biochemical reactions. The metamorphic insects have developed an effective antioxidant defense system to overcome the harsh conditions. Lots of work has been conducted to explore the antioxidant net-working mechanisms. However, strategies of antioxidant defense in diapause onion fly pupae are presently unknown.

The important protective enzymes of antioxidant system in insects include SOD, CAT and GPx. SOD is the first enzyme scavenging superoxide anions to protect the internal stability. CAT and GPx are the key enzymes to convert the resulting free radicals H_2O_2 to water and oxygen. Generally, the average activities of these three protective enzymes in diapause pupae were relatively lower than those in non-diapause individuals, suggesting that metabolic rates were lower during diapause period. In *C. pipens*, the expression level of *sod*-2 (a homolog of *Cu/Zn-SOD*) was significantly higher in young diapause females than in their non-diapause counterparts. Suppression of *sod*-2 could shorten the survival time of adults (Sim and Denlinger, 2011). In this study, it was worth noting that the Cu/Zn-SOD activity was significantly increased at pre-diapause phase of WD pupa, and the post-diapause phase of SD pupa, indicating more ROS were generated during the two developmental phases. No significant change in Cu/Zn-SOD activity in SD pupae at the pre-diapause phase suggested that it was not Cu/Zn-SOD but other antioxidant enzymes that might play a crucial role during this phase. Relatively lower activities of Cu/Zn-SOD and MnSOD at the diapause maintenance phase (developmental stages 4 – 5) were consistent with the depressed metabolic rate during the diapause. Variations of SOD activity in *D. antiqua* might be linked to different functions as well as different cellular localizations. MnSOD is localized in mitochondria and peroxisomes, while Cu/Zn-SOD is mainly localized in the cytoplasm or extracellular. Interestingly, in the dauer stage of *C. elelgans*, a similar state of insect diapause, the expression levels of two orthologs of Cu/Zn-SOD were significantly increased (Honda *et al.*, 2008). Therefore, further investigation of tissue-specific expression patterns of these SOD enzymes may help explain their functions during diapause development of *D. antiqua*.

An increased average CAT activity in ND pupae might balance increased mitochondrial hydrogen peroxide production. In non-diapause pupae, higher respiration rate and higher oxygen consumption might have resulted in increasing ROS production, however it was opposite in diapause pupae. A similar result was also observed in non-diapause larvae and corresponding pupae of the European corn borer (*Ostrinia nubilalis*) (Jovanović-Galović *et al.*, 2004). In pre-diapause phase, the gene expression level of CAT in *C. pipiens* diapause females was also dramatically higher than that in non-diapause one (Sim *et al.*, 2015), which was corroborated by our findings. Similar activity pattern of Cu/Zn-SOD and CAT in onion fly pupae suggested that they cooperated closely to defend peroxide stresses. Previous study showed that the H_2O_2 level was remarkably higher in diapause *B. mori* eggs than in non-diapause individuals, and it is hypothesized that H_2O_2 might be involved in the termination of diapause egg (Zhao and Shi, 2010). In this study, higher SOD activity and lower CAT activity at the post-diapause phase would result in the increase of H_2O_2 level, which might also play indirect roles in the regulation of diapause termination, just like in diapause *B. mori* egg.

As another important component of the oxygen scavenging system, GR catalyzes the reduction of GSSH to GSH, which is a crucial molecule in maintaining the reducing environment of the cell (Deponte, 2013). Therefore, the GSH/GSSG ratio is a dynamic indicator of oxidative stress, and a high ratio is indicative of reducing condition. A recent study demonstrated that the GSH/GSSG ratio was an important regulatory factor at the initiation, maintenance and termination of the diapause of *B. mori* (Zhao *et al.*, 2014) and concluded that GSH/GSSG shift was partly from the need of diapause state changes. Compared with ND pupae, both SD and WD pupae had lower GSH/GSSG ratio and the reverse variation trend in the activities of GP_X and GR during the diapause maintenance phase (developmental stages 3 – 5), suggesting a redox status shift in the glutathione redox cycle during the diapause process. Stronger oxidation of GSH and weaker reduction of GSSG may result in the lower GSH/GSSG ratio, indicating that GR and/or other enzymes contributed to the reduction of GSSG. Lower GSH/GSSG ratios at pre-diapause phase and diapause maintenance phase, higher ratios at post diapause phase suggested that the glutathione redox-oxidative status dynamically varied during pupal diapause development of onion fly.

From the present findings, we concluded that the overall oxidant and antioxidant status were significantly different in ND, SD and WD pupae. Higher activities of Cu/Zn-SOD, CAT and MnSOD in pre-diapause phase, and higher activities of GPx and MnSOD in post-diapause phase along with the variation of GSH/GSSG ratio indicated that the oxidative shift resulted from a higher respiration rate and the consequences of morphological changes.

References

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72(1 – 2): 248 – 254.

Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, Gems D, 2011. Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radic. Biol. Med.*, 51(8): 1575 – 1582.

Costantini D, 2014. Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology. Springer-Verlag Berlin Heidelberg. 1 – 38.

De Block M, Stoks R, 2008. Compensatory growth and oxidative stress in a damselfly. *Proc. Biol. Sci.*, 275(1636): 781 – 785.

Deponte M, 2013. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim. Biophys. Acta*, 1830 (5): 3217 – 3266.

Dmochowska-Ślęzak K, Fliszkiewicz M, Giejdasz K, Żółtowska K, 2015. The antioxidant system in diapausing and active red mason bee *Osmia bicornis*. *Physiol. Entomol.*, 40(1): 82 – 89.

Fu DY, Si FL, Chen B, Xu YL, Hao YJ, 2016. Transcriptome-guided identification of the cuticular protein gene family of *Delia antiqua* (Diptera: Anthomyiidae) and expression analysis under non- and winter-diapause conditions. *Acta Entomol. Sin.*, 59 (2): 172 – 184. [付丹影, 司风玲, 陈斌, 徐燕玲, 郝友进, 2016. 基于转录组的葱蝇表皮蛋白基因家族的鉴定及在非滞育和冬滞育条件下的表达分析. 昆虫学报, 59(2): 172 – 184]

Hao YJ, Zhang YJ, Si FL, Fu DY, He ZB, Chen B, 2016. Insight into the possible mechanism of the summer diapause of *Delia antiqua* (Diptera: Anthomyiidae) through digital gene expression analysis. *Insect Sci.*, 23(3): 438–451.

Hernández-García D, Wood CD, Castro-Obregón S, Covarrubias L, 2010. Reactive oxygen species: a radical role in development? *Free Radic. Biol. Med.*, 49(2): 130–143.

Honda Y, Tanaka M, Honda S, 2008. Modulation of longevity and diapause by redox regulation mechanisms under the insulin-like signaling control in *Caenorhabditis elegans*. *Exp. Gerontol.*, 43: 520–529.

Jovanović-Galović A, Blagojević DP, Grubor-Lajsić G, Worland R, Spasić MB, 2004. Role of antioxidant defense during different stages of preadult life cycle in European corn borer (*Ostrinia nubilalis*, Hubn.): diapause and metamorphosis. *Arch. Insect Biochem. Physiol.*, 55(2): 79–89.

MacRae TH, 2010. Gene expression, metabolic regulation and stress tolerance during diapause. *Cell Mol. Life Sci.*, 67(14): 2405–2424.

Missirlis F, Phillips JP, Jäckle H, 2001. Cooperative action of antioxidant defense systems in *Drosophila*. *Curr. Biol.*, 11(16): 1272–1277.

Mittapalli O, Neal JJ, Shukle RH, 2007. Tissue and life stage specificity of glutathione S-transferase expression in the Hessian fly, *Mayetiola destructor*: implications for resistance to host allelochemicals. *J. Insect Sci.*, 7(1): 1–13.

Munks RJ, Sant' Anna MR, Grail W, Gibson W, Igglesden T, Yoshiyama M, Lehane SM, Lehane MJ, 2005. Antioxidant gene expression in the blood-feeding fly *Glossina morsitans morsitans*. *Insect Mol. Biol.*, 14(5): 483–491.

Nabizadeh P, Kumar TJ, 2011. Fat body catalase activity as a biochemical index for the recognition of thermotolerant breeds of mulberry silkworm, *Bombyx mori* L. *J. Therm. Biol.*, 36(1): 1–6.

Rajarapu SP, Mamidala P, Herms DA, Bonello P, Mittapalli O, 2011. Antioxidant genes of the emerald ash borer (*Agrilus planipennis*): gene characterization and expression profiles. *J. Insect Physiol.*, 57(6): 819–824.

Sim C, Denlinger DL, 2011. Catalase and superoxide dismutase-2 enhance survival and protect ovaries during overwintering diapause in the mosquito *Culex pipiens*. *J. Insect Physiol.*, 57(5): 628–634.

Sim C, Kang DS, Kim S, Bai X, Denlinger D, 2015. Identification of FOXO targets that generate diverse features of the diapause phenotype in the mosquito *Culex pipiens*. *Proc. Natl. Acad. Sci. USA*, 112(12): 3811–3816.

Zhao LC, Shi LG, 2010. Metabolism of hydrogen peroxide between diapause and non-diapause eggs of the silkworm, *Bombyx mori* during chilling at 5℃. *Arch. Insect Biochem. Physiol.*, 74(2): 127–134.

Zhao LC, Hou YS, Sima YH, 2014. Changes in glutathione redox cycle during diapause determination and termination in the bivoltine silkworm, *Bombyx mori*. *Insect Sci.*, 21(1): 39–46.

葱蝇非滞育、夏滞育和冬滞育蛹体内抗氧化酶活性的比较分析

郝友进[*]，郭 强，陈 斌

（重庆师范大学昆虫与分子生物学研究所，媒介昆虫重庆市重点实验室，重庆 401331）

摘要：【目的】通过测定并比较分析抗氧化酶活性及谷胱甘肽氧化还原状态，探讨葱蝇 *Delia antiqua* 非滞育、夏滞育和冬滞育蛹体内抗氧化系统的差异。【方法】取葱蝇非滞育、夏滞育和冬滞育蛹，分别测定铜锌超氧化物歧化酶（Cu/Zn-SOD）、锰超氧化物歧化酶（MnSOD）、过氧化氢酶（CAT）、谷胱甘肽过氧化物酶（GPx）和谷胱甘肽还原酶（GR）活性及还原型谷胱甘肽（GSH）和氧化性还原型谷胱甘肽（GSSG）含量及 GSH/GSSG 比随发育时间的变化；并对各抗氧化因子进行了典型判别分析。【结果】5 种抗氧化酶的活性在整个蛹期是动态变化的。与非滞育蛹和夏滞育蛹相比，处于滞育前期的冬滞育蛹具有较高的 Cu/Zn-SOD 酶活性，但处于夏滞育及冬滞育的维持期和滞育后期的滞育蛹体内 Cu/Zn-SOD 酶活性则明显低于同一发育时期的非滞育蛹。在整个蛹期，非滞育蛹体内 MnSOD 酶活性显著高于夏滞育和冬滞育蛹，而在夏滞育和冬滞育蛹之间则无明显差异。比较两种酶活性则发现同一发育时期的 MnSOD 平均酶活性明显高于 Cu/Zn-SOD 酶活性。在头外翻之前，滞育蛹体内 CAT 酶活性高于非滞育蛹，但处于滞育维持期和后期的蛹体内 CAT 酶活性则低于非滞育蛹。无论在非滞育蛹还是滞育蛹中，GPx 和 GR 酶活性变化基本上呈相反的趋势。典型判别分析进一步表明葱蝇蛹体内的抗氧化系统具有发育时期和滞育类型特异性。【结论】非滞育蛹与夏滞育和冬滞育蛹体内的氧化还原状态存在明显差异。滞育前期和滞育后期的蛹体内较高的抗氧化酶活性和较低的 GSH/GSSG 比提示氧化状态的变化与高的呼吸速率及发育过程密切相关。

关键词：葱蝇；滞育；抗氧化酶；氧化胁迫；谷胱甘肽氧化还原循环

中图分类号：Q966　**文献标识码：**A　**文章编号：**0454-6296(2018)03-0263-08

（责任编辑：赵利辉）